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Effect of Chemical Perturbation with NaSCN on Receptor–Estradiol Interaction. A New Exchange Assay at Low Temperature†

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ABSTRACT: When 0.5 M sodium thiocyanate is added to uterine cytosol previously labeled with excess [^{3}H]-17β-estradiol, no change can be detected in the steady-state cytosol concentration of [^{3}H]estradiol–receptor complex for at least 20 h at 4 °C. However, the rate of exchange of bound estradiol in the presence of NaSCN was found to be substantially higher than that in the absence of the chaotropic salt. In the presence of NaSCN, the dissociation rate of the complex increases about 10-fold (K_{-1}^{SCN} = 1.10 × 10^{-2} min^{-1} vs. K_{-1} = 1.07 × 10^{-3} min^{-1}) while the rate of association increases about 2-fold (K_1^{SCN} = 1.2 × 10^7 min^{-1} M^{-1} vs. K_1 = 7.4 × 10^6 min^{-1} M^{-1}). The K_d changes 6.4-fold (K_d^{SCN} = 9 × 10^{-10} M vs. K_d = 1.4 × 10^{-10} M) with no decrease in the number of binding sites as shown by Scatchard plots of saturation experiments. This effect of NaSCN can be exploited to assay preformed estrogen–receptor complex by exchange with [^{3}H]estradiol at low temperature. When the sample containing preformed complex is incubated overnight (16 h) at 4 °C with excess [^{3}H]estradiol in the presence of 0.5 M NaSCN, there is a quantitative exchange of nonlabeled for labeled estradiol without loss of binding sites. Hormonal steroids other than estrogens do not interfere, and the exchanged estradiol is bound with high affinity. Precision, accuracy, and linearity of the method are highly satisfactory.

Specific ion effects on macromolecules have been recognized since Hofmeister (1888) noted that salts differ greatly in their ability to salt out proteins. A wide variety of salt effects on biological systems have since been found, and specifically those ions that are the most effective precipitants lead to folding, coiling, and aggregation while those least effective promote unfolding, extension, and dissociation. The latter ions have been termed "chaotropic" (tending to disorder) by Hamaguchi & Geiduschenk (1962). The effects of chaotropic ions have been interpreted as weakening or disrupting the hydrophobic bonding and at the same time as affecting hydrogen bonding and electrostatic attractions (Dandliker et al., 1967; Dandliker & de Saussure, 1971). Such salts are known to increase the solubility of nonpolar molecules in water, and thermodynamic evidence suggests that they make water more "disordered" or lipophilic (Hatefi & Haustein, 1969; Haustein et al., 1971).

Contrary to agents like urea, guanidine hydrochloride, or detergents, chaotropic agents produce dissociation of proteins at concentrations which do not cause major shifts in protein conformation (Swayer & Puckridge, 1973).

Recently, we have used chaotropic salts to prevent the age-dependent aggregation of estradiol receptor in cytosol. We have found that sodium thiocyanate up to 0.5 M is compatible with a stable estradiol–receptor complex during sucrose gradient centrifugation; however, the maximum permissible concentration is 0.1 M during Sephadex G-100 and G-200 chromatography. In fact, at higher thiocyanate concentrations the estradiol–receptor complex dissociates (Sica et al., 1976). This strongly suggests that chaotropic salt concentrations higher than 0.1 M weaken the binding of estradiol to the receptor. In this paper, we have investigated the kinetics of association and dissociation of the estradiol–receptor complex in the presence of sodium thiocyanate and have found that both association and dissociation rates are increased. Furthermore, we have investigated the possibility of using this increased turnover to facilitate the exchange of radioactive estradiol into binding sites previously filled with "cold" estradiol.

Materials and Methods

Materials. All reagents were of analytical grade. NaSCN (ACS) was purchased from C. Erba. [6,7-$^3H_2$]-17β-Estradiol

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([^{3}H]-17β-E_2)[1] (sp act. 60 Ci/mmol; New England Nuclear) was 97% pure at the time of the experiments. Tris and EDTA disodium salt were purchased from Sigma Chemical. Dithiothreitol was from Calbiochem; charcoal and Norit A were from Matheson Coleman and Bell, Norwood, OH; Dextran T 70 was from Pharmacia.

Radioactivity Assay. Aqueous samples (0.1–1 mL) were added to 5 mL of Insta Gel liquid scintillation cocktail (Packard) in glass scintillation vials, and the radioactivity was measured in a Beckman LS-3150 T counter with 50% efficiency.

Protein Assay. Protein determination was performed by the Bio-Rad protein assay based on the work of Bradford (1976). Thiol groups, Tris, and EDTA do not interfere with this assay.

Preparation of Cytosol. All operations were carried out at +4 °C, either in a cold room or in refrigerated centrifuges. Immature calf uteri weighing not more than 30 g were collected at the local slaughterhouse as soon as the animals were killed and kept in plastic bags buried in crushed ice while rushed to the laboratory. The uteri were stripped of connective tissue and frozen in liquid nitrogen. Before use they were pulverized in a mortar and homogenized in 4 volumes of TED buffer (0.01 M Tris-HCl, 1 mM EDTA, and 1 mM dithiothreitol, pH 7.5) by means of an Ultraturrax homogenizer (Janke and Kunkel, Model TP 18/2) in four runs of 30 s each, with 60-s intervals. The homogenate was centrifuged for 45 min in a Beckman-Spinco L2-65 ultracentrifuge at 150000*g* and the decanted supernatant collected.

Assay of Cytoplasmic Estradiol Receptor Activity. The assay of estradiol receptor in the cytosol was performed as follows unless otherwise indicated.

Aliquots of cytosol were incubated with [6,7-$^3H_2$]estradiol (5 ng/mL) at 4 °C for 2 h, a sufficient time to reach equilibrium. After incubation, separation of free from macromolecule-bound [6,7-$^3H_2$]estradiol was accomplished by adsorption of free hormone to an equal volume of a slurry of dextran-coated charcoal (DCC = 1% Norit A and 0.05% dextran in TED) for 15 min at 4 °C, followed by centrifugation at 1500*g* for 10 min. Aliquots of 0.5–1 mL of the supernatant (2000*g*, 15 min) were added to 6 mL of Insta Gel, and radioactivity was measured. The correction for nonspecific binding was determined in a parallel incubation in which a 100-fold excess of unlabeled estradiol had been added. The difference in the amount of bound ligand in the presence and absence of cold estradiol was taken as a measure of the specific high-affinity binding. For avoidance of casual differences in the binding assay due to the presence in certain samples of NaSCN, all assays were performed in the presence of 0.1 M NaSCN by either adding 0.1 M NaSCN to the samples without salt or diluting the samples to this NaSCN concentration before adding the DCC suspension.

Exchange Assay. As described under Results, optimum conditions for achieving complete exchange of estrogen-filled sites involve incubation of cytosol at 4 °C for 14–18 h in the presence of 0.5 M NaSCN and excess radioactive estradiol. Cytosol was preincubated 4 h at 4 °C with saturating concentrations of nonradioactive estradiol (5 ng/mL) and then added to the pellet obtained after centrifugation (2000*g*; 15 min) of an equal volume of DCC. The cytosol–DCC suspension was kept for 15 min at 4 °C. After centrifugation, aliquots of the charcoal-stripped cytosol were incubated with

[1] Abbreviations used: [^{3}H]E_2, [6,7-$^3H_2$]estradiol; 17β-E_2, 17β-estradiol; [^{3}H]-17β-E_2, [6,7-$^3H_2$]-17β-estradiol.

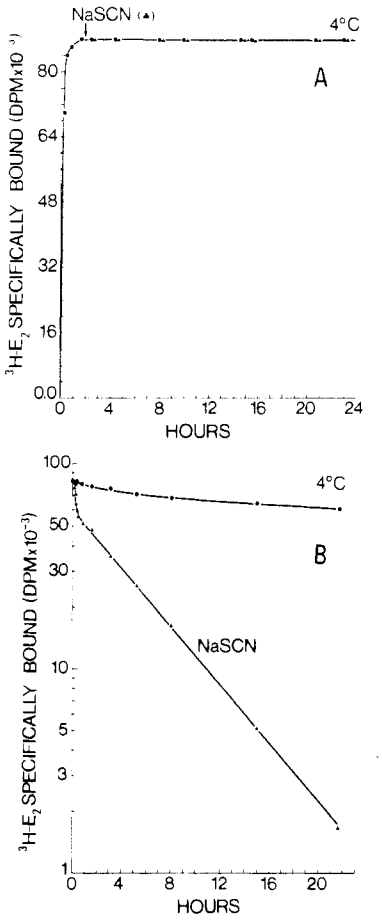

FIGURE 1: (A) Stability of estradiol–receptor complex in the presence of 0.5 M NaSCN. Uterine cytosol was incubated with [6,7-$^3H_2$]-estradiol (5 ng/mL) at 4 °C. At the indicated time, aliquots of 0.2 mL of incubating mixture were pipetted into 0.4 mL of ice-cold TED buffer and the binding assay was performed with the DCC method described under Materials and Methods. After 90 min, 0.5 M NaSCN (final concentration) was added to part of mixture. Specific binding sites in the absence (●) and in the presence (▲) of NaSCN per 0.1 mL of cytosol. (B) Dissociation rate of estradiol–receptor complex in the presence of 0.5 M NaSCN. Uterine cytosol was incubated with a saturating concentration (5 ng/mL) of [6,7-$^3H_2$]estradiol at 4 °C. At the end of incubation a 1000-fold excess of unlabeled estradiol was added and the incubation was continued for 16 h. [^{3}H]E_2 bound in the presence (▲) or in the absence (●) of 0.5 M NaSCN.

various amounts of radioactive (total binding) or radioactive estradiol plus a 100-fold excess of cold estradiol (nonspecific binding) in the presence of indicated NaSCN concentrations. The samples were allowed to stand for 14–18 h at 4 °C and then were diluted with ice-cold TED buffer to 0.2 M NaSCN. After an additional incubation (usually 1 h at 4 °C, required to reach the equilibrium at the new salt concentration), the free hormone was adsorbed to DCC as described in the previous section. All the assays were performed in duplicate or triplicate. Variations among the single determinations were below 5%.

Results

Stability of Estradiol–Receptor Complex in the Presence of Thiocyanate. The uterine supernatant preparation used in this section contained a high proportion of high-affinity estradiol binding sites. Equilibrium between estradiol and the receptor was established by incubating uterine cytosol with radioactive estradiol at 4 °C. At the indicated time (Figure 1A) portions of the incubation mixture were withdrawn and the binding assay was performed as described under Material and Methods. When equilibrium was reached (90–120 min),

Table I: Parameters of Estradiol Binding

parameters	control	NaSCN
first phase		
$10^{-6}K_1$ (M^{-1} min^{-1})	7.4	12.7
$10^{3}K_1$ (min^{-1})	1.1	11.4
$10^{10}K_d$ (M)	1.4	9.0
second phase		
$10^{-6}K_1$ (M^{-1} min^{-1})	4.3	5.2
$10^{3}K_1$ (min^{-1})	0.098	2.7
$10^{10}K_d$ (M)	0.23	5.1

the mixture was divided into two parts and 0.5 M NaSCN was added to one of them. The addition of thiocyanate does not interfere with the stability of the estradiol–receptor complex for at least 20 h. A parallel experiment was performed to assess the amount of nonspecific binding (less than 5% of total binding), and this value was subtracted from the total binding.

The data shown in Figure 1A clearly show that relatively high concentrations of thiocyanate are compatible with a stable estradiol–receptor complex. However, the maximum permissible concentration during Sephadex chromatography (Sica et al., 1976) or during experiments requiring extensive dialysis (data not shown) is 0.1 M. Under these experimental conditions, higher concentrations of NaSCN dissociate the hormone–receptor complex. Since the formation of the steroid–protein complex is regulated by an equilibrium subject to the law of mass action, any change in the concentration of unbound steroid would alter the percentage of the bound portion. The behavior of the estradiol–receptor complex during the chromatographic and dialysis experiments can be explained by thiocyanate increasing both the association and dissociation rates of the hormone to the receptor. In fact, in these experiments the dissociated hormone is continuously removed, remarkably affecting the concentration of unbound steroid and hence the percentage of the bound portion. On the contrary, the free hormone concentration is unchanged during the incubation illustrated in Figure 1A and therefore the amount of hormone–receptor complex is unaffected.

Rate of Dissociation of Estradiol from the Steroid–Receptor Complex in the Presence of Thiocyanate. The process of dissociation of the [6,7-$^3H_2$]estradiol–receptor complex was rendered first order by adding a 10^3-fold excess of unlabeled estradiol in a negligible volume to the cytosol brought to equilibrium by preincubation for 4 h at 4 °C with saturating concentrations (5 ng/mL) of radioactive estradiol. When plotted as a first-order reaction, the dissociation of estradiol–receptor complex at 4 °C was very slow and biphasic (Figure 1B), showing the well established two-step, first-order kinetics pattern (Best-Belpomme et al., 1970; Mester et al., 1970; Sanborn et al., 1971; Erdos et al., 1971; Truong & Baulieu, 1972; Sala-Trepat & Reti, 1974; Vallet-Strouve et al., 1976; McGormack & Glasser, 1976), but in the presence of 0.5 M NaSCN both components are faster. The data were analyzed on the basis of the reaction equation

$$E + R \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ER \qquad (1)$$

where E refers to estradiol and R to receptor.

As shown in Table I, in the presence of thiocyanate there is a 10-fold increase ($K_{-1}^{SCN} = 11.4 \times 10^{-3}$ min^{-1} vs. $K_{-1} = 1.1 \times 10^{-3}$ min^{-1}) of the faster component of the dissociation rate while the slower part is 27-fold faster than the control ($K_{-1}^{SCN} = 2.7 \times 10^{-3}$ min^{-1} vs. $K_{-1} = 0.098 \times 10^{-3}$ min^{-1}).

Rate of Association of Estradiol with the Receptor in the Presence of Thiocyanate. Figure 2 shows the data obtained

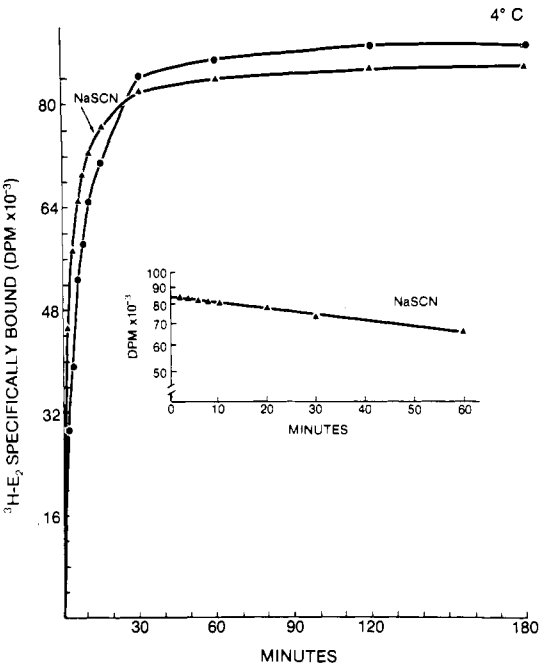

FIGURE 2: Association rate of estradiol to the receptor in the absence (●) or in the presence (▲) of 0.5 M NaSCN. Insert: inactivation rate of "free" receptor in the presence of 0.5 M thiocyanate.

with parallel incubations of uterine cytosol with a given concentration of ^3H-labeled steroid with or without 0.5 M NaSCN. The nonspecific binding measured in the presence of a 100-fold excess of cold estradiol was subtracted from the total binding. At the indicated time, 0.2-mL portions were withdrawn and assayed for binding with the DCC method.

Preliminary experiments showed that the receptor without bound hormone was affected by the presence of NaSCN in a manner similar to the hormone–receptor complex and that the protein could be stabilized completely by estradiol. In fact, when the cytosol was preincubated for various times at 4 °C before addition of [6,7-$^3H_2$]estradiol, inactivation of the free receptor could clearly be demonstrated (Figure 2, insert). Detailed measurements were made of the binding of [6,7-$^3H_2$]estradiol to the receptor during 180 min of incubation at 4 °C. The value for total receptor concentration was determined with a saturating concentration of [6,7-$^3H_2$]estradiol in the absence of thiocyanate. The data shown in Figure 2 clearly indicate that sodium thiocyanate alters the rate but very little the extent of estradiol binding to receptor. Similar results were obtained in several experiments. The association rate was calculated according to Munck (1976).

The binding was found to take place in two phases. Values for these phases are reported in Table I.

The table includes also the dissociation rate constants (K_{-1}) observed in the experiment described in the previous section and the consequently calculated dissociation equilibrium constants, $K_d = K_{-1}/K_1$ ($K_d^{SCN} = 9 \times 10^{-10}$ M vs. $K_d = 1.4 \times 10^{-10}$ M). This value is not dissimilar from the calculated dissociation equilibrium constants obtained in the presence and absence of thiocyanate and shown in Figure 3 ($K_d^{SCN} = 8 \times 10^{-10}$ M vs. $K_d = 3 \times 10^{-10}$ M).

Use of Thiocyanate to Measure Estradiol-Filled Receptor Sites. In the absence of thiocyanate the rate of estradiol dissociation from the binding protein at 4 °C is so slow that only minimal exchange occurs during the time of assay. Because of this minimal exchange, determinations of the total concentration of estrogen receptor in direct assays utilizing [6,7-$^3H_2$]estradiol may be erroneous when estradiol binding

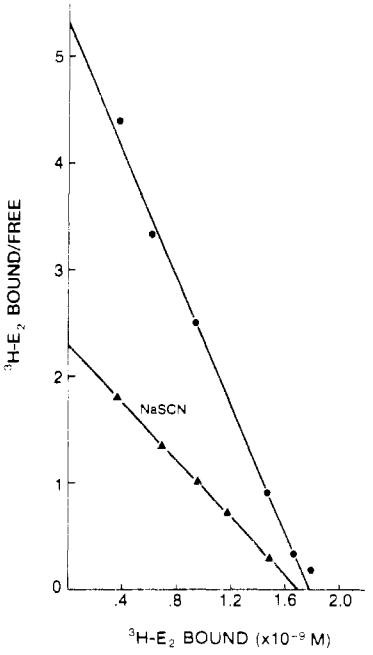

FIGURE 3: Scatchard plot of the receptor binding to estradiol with (▲) or without (●) 0.5 M NaSCN.

sites are partially or completely filled with cold hormone.

Because of the obvious importance of being able to assess *total* receptor content of the cell, there have been numerous attempts to determine optimal conditions for increasing the rate of dissociation of the estrogen–receptor complex without loss of binding activity. All of these attempts are based on careful and controlled increases of temperature (Anderson et al., 1972; Katzenellenbogen et al., 1973; Chamness et al., 1975; Zava et al., 1976; Sutherland & Baulieu, 1976). Considerations of the above methods and results point out very clearly that a delicate balance must be struck between the noxious temperature–time factor and the achievement of a sufficiently rapid rate of exchange for practical purpose. The estradiol receptor is quite sensitive to higher temperature and, especially in the case of crude preparations, there is a definite possibility of proteolytic destruction of the receptor.

Results described in the preceding sections have shown that thiocyanate increases both the dissociation and association rates of the estradiol–receptor complex at 4 °C and that the complex is quite stable for several hours. Therefore, thiocyanate can be used to exchange radioactive estradiol with cold estradiol when cytoplasmic binding sites are filled with the unlabeled hormone and to determine the total concentration of binding sites, regardless of what fraction of the sites is filled at the time of analysis and without loss of binding activity.

Optimization of Assay Parameters. (*a*) *Exchange as a Function of NaSCN Concentration.* As shown in Figure 4, when uterine cytosol presaturated with cold estradiol is incubated at 4 °C with radioactive hormone in the presence of increasing amounts of NaSCN, the cold steroid is actually exchanged with the labeled estradiol. The lowest NaSCN concentration that gives the maximum effect is 0.5 M.

(*b*) *Exchange as a Function of* [6,7-$^3H_2$]*Estradiol Concentration.* The optimal concentration of labeled ligand that effects saturation of the binding protein is 15 nM (Figure 5).

As discussed by several authors (Katzenellenbogen et al., 1973; Sutherland & Baulieu, 1976), many factors influence the choice of the concentration of estradiol. The concentration of the hormone must be high enough to ensure saturation of receptor sites and also be present in a large enough excess to

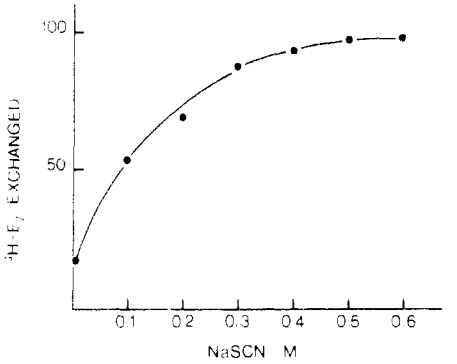

FIGURE 4: Exchange of 17β-E$_2$ vs. [^{3}H]-17β-E$_2$ as a function of NaSCN concentration. Uterine cytosol was saturated with 17β-estradiol. Excess hormone was then adsorbed on DCC. The complex was then incubated for 16 h at 4 °C in the presence of [^{3}H]-17β-estradiol (15 nM) and different concentrations of NaSCN.

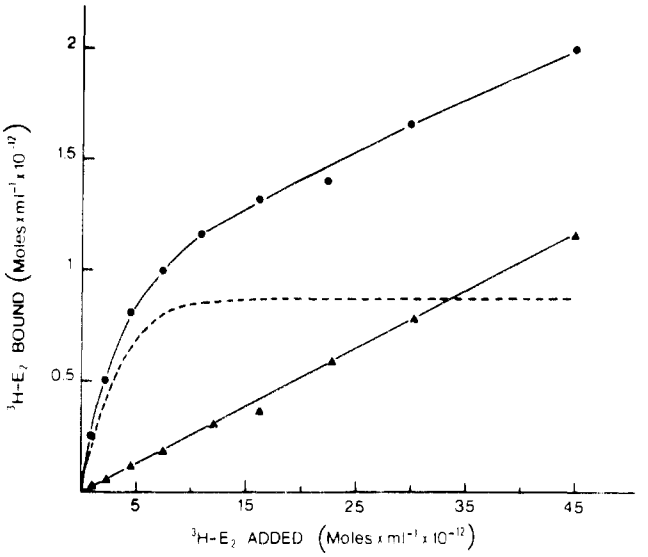

FIGURE 5: Exchange of 17β-E$_2$ vs. [^{3}H]-17β-E$_2$ as a function of [^{3}H]-17β-E$_2$ concentration. Uterine cytosol presaturated with 17β-estradiol was incubated for 16 h at 4 °C in the presence of 0.5 M NaSCN and different concentrations of [^{3}H]-17β-estradiol alone (total binding, ●) or [^{3}H]-17β-estradiol plus cold 17β-estradiol at 100 times the concentration of the labeled hormone (nonspecific binding, ▲). (---) specific binding obtained by subtracting nonspecific from total binding.

decrease the dilution of the specific radioactivity of the labeled ligand when the endogenous hormone dissociates from the receptor sites during the exchange. On the other hand, a too high concentration of radioactive ligand would increase the level of nonspecific binding and would reduce the assay sensitivity.

From our data, a concentration of hormone (20 nM) more than 25-fold higher than the amount required to fill all the binding sites (0.75 nM; see Figure 5) gives a very small dilution of specific radioactivity (less than 4%) which in most cases can be neglected. This concentration is still compatible with an acceptable level of nonspecific binding.

(*c*) *Rate of Exchange.* Figure 6 shows that there is a saturable exchange of radioactive estradiol for unlabeled estradiol within 16 h at 4 °C. More than 95% of the hormone has exchanged with no measurable loss of binding sites.

(*d*) *Linearity, Accuracy, and Precision.* The exchange assay is a linear function of the amount of cytosol (Figure 6, insert).

The accuracy, the precision, and the reproducibility of the method are very high (Table II). Specific binding of [^{3}H]-estradiol assayed by direct incubation of 10 samples of uterine

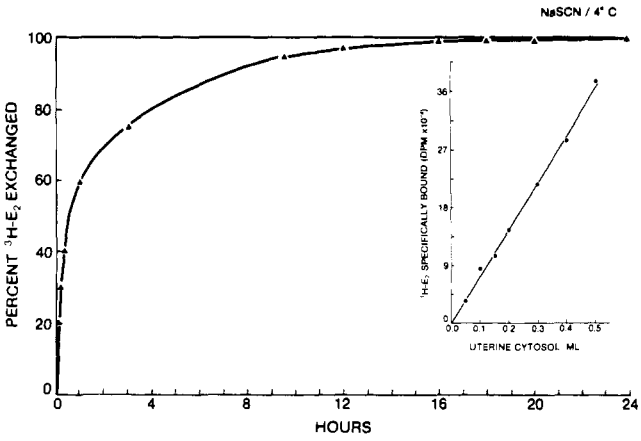

FIGURE 6: Exchange rate of 17β-E_2 vs. [^{3}H]-17β-E_2. Uterine cytosol saturated with 17β-E_2 was incubated at 4 °C with 0.5 M NaSCN and 15 nM [^{3}H]-17β-estradiol. Insert: linearity of exchange assay. Different concentrations of cytosol were saturated with 17β-estradiol and exchanged for 16 h at 4 °C with 15 nM [^{3}H]-17β-estradiol. Nonspecific binding was determined in the presence of a 100-fold excess of cold hormone and subtracted from the total binding.

Table II: Accuracy and Precision of the NaSCN Exchange Method

sp binding of [^{3}H]E_2 by uterine cytosol incubd with [^{3}H]E_2 for 2 h at 4 °C			sp binding of [^{3}H]E_2 by uterine cytosol presatd with E_2 and then incubd with [^{3}H]E_2 in the presence of NaSCN for 16 h at 2 °C		
assay no.	cpm/50 μL of cytosol	limits (cpm/50 μL of cytosol)	assay no.	cpm/50 μL of cytosol	limits (cpm/50 μL of cytosol)[a]
1	18 588		1	17 923	
2	18 157		2	18 415	
3	15 363	$\bar{m}$ = 17456	3	16 333	$\bar{m}$ = 17093
4	16 821	S_x = 1053	4	17 615	S_x = 1283
5	16 860	S_x' = 999	5	19 393	S_x' = 1217
6	17 488	$S_{\bar{x}}$ = 333	6	16 815	$S_{\bar{x}}$ = 405
7	17 124	$S_{\bar{x}}'$ = 316	7	15 941	$S_{\bar{x}}'$ = 384
8	17 243		8	16 318	
9	19 131		9	15 003	
10	17 791		10	17 810	

[a] $\bar{m}$ = mean; S_x = standard deviation by the $n-1$ method; S_x' = standard deviation by the n method; $S_{\bar{x}}$ = standard error of the mean by the $n-1$ method; $S_{\bar{x}}'$ = standard error of the mean by the n method; Δ = difference between the means = 363; t = t statistic = −0.039; DF = degrees of freedom = 18.

cytosol exhibits no significant difference (t = 0.39; DF = 18) when compared to the results of assays performed by the NaSCN exchange method.

(*e*) *Specificity of Exchange.* Specificity of the NaSCN-induced exchange of labeled estradiol for cold hormone in uterine cytosol is presented in Table III. The exchange occurs only in the presence of estrogens (estradiol, estrone, and estriol). Testosterone, cortisone, deoxycorticosterone, and progesterone are ineffective.

Discussion

When 0.5 M sodium thiocyanate is added to uterine cytosol previously labeled with an excess of [6,7-$^3H_2$]estradiol, no change can be detected in the steady-state cytosol concentration of [^{3}H]-17β-estradiol–receptor complex for at least 20 h at 4 °C. However, the rate of exchange of bound estradiol in the presence of NaSCN was found to be substantially higher than that in the absence of the chaotropic salt. In the presence of NaSCN the dissociation rate of the complex increases and presents a characteristic biphasic pattern. The dissociation

Table III: Specificity of NaSCN-Induced Exchange of [^{3}H]E_2 for E_2 in Uterine Cytosol

cold hormonal steroid added to exchange system	% [^{3}H]E_2 complex[a] in presence of competitor	cold hormonal steroid added to exchange system	% [^{3}H]E_2 complex[a] in presence of competitor
none	100	testosterone	98
17β-steroid	2	cortisone	116
estrone	46	deoxycorticosterone	96
estriol	72	progesterone	91

[a] Mean of triplicate determinations.

of the faster component shows a 10-fold increase while the slower component is 27 times faster than the control.

The rate of association increases about twofold. Accurate measurements of the association rate of estradiol with the cytoplasmic receptor were complicated by inactivation of the receptor. Since, in any case, this inactivation was very low, it had very little effect on the estimated association rate constant K_1.

The K_d in the presence of NaSCN changes slightly (6.4-fold), as shown by Scatchard plots of saturation experiments.

On the basis of these properties, a simple method has been worked out by which receptor bound to nonradioactive hormone can be assayed in uterine cytosol by direct incubation at 4 °C overnight (16 h) in the presence of a saturating amount of [^{3}H]estradiol and 0.5 M NaSCN. The method is reproducible, accurate, and precise.

In a previous work, Anderson et al. (1972) carried out the estradiol exchange assay of nuclear receptor by direct incubation of a nuclear fraction at 37 °C for 1 h. However, noting the several disadvantages of the Anderson et al. (1972) method, i.e., high nonspecific [^{3}H]estradiol binding, instability of the receptor at 37 °C, and variable replication, Zava et al. (1976) have proposed a method in which nuclear receptor is first extracted with 0.6 M KCl and precipitated with protamine sulfate and the precipitate is subjected to ligand exchange with radiolabeled estradiol at 37 °C for 2.5 h. This test for nuclear receptor is an application of a method previously developed by the same group (Chamness et al., 1975) to assay preformed endogenous estrogen–receptor complex in cytosol. After a very detailed investigation, Katzenellenbogen et al. (1973) suggested that exchange assays of cytoplasmic estradiol-binding filled sites be conducted by incubation of cytosol for 18–24 h at 25–30 °C. They noticed a significant loss of binding sites at 37 °C, even in the presence of a large excess of hormone.

The unquestionable advantage of our method is that it can be performed at low temperature and therefore decreases the risk of temperature inactivation of proteolytic destruction of the receptor.

Finally, the NaSCN exchange method has already proven very helpful in the specific hormonal elution of receptor from estradiol-containing agarose supports used in affinity chromatography (Bresciani et al., 1978; Sica & Bresciani, 1979). The overnight incubation with NaSCN at 4 °C of receptor adsorbed to an affinity support achieves a more efficient and less inactivating elution than the previous method of increasing the temperature to 30 °C for 20 min (Sica et al., 1976).

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Estimation of the Lateral Distribution of Molecules in Two-Component Lipid Bilayers†

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ABSTRACT: A new formalism to investigate the lateral distribution of molecules in lipid bilayers has been developed, and the results have been applied to the case of phosphatidylcholine mixtures. It is demonstrated that the experimental phase diagrams for these mixtures can provide the necessary information to generate computer-simulated bilayers with the desired molecular interactions and lattice constraints. Analysis of these computer-generated lipid bilayers allows calculation of the number of contacts between like and unlike molecules, the average size and number of compositional clusters, and the pair correlation functions. The results of this analysis provide a full quantitative description of the molecular organization of phosphatidylcholine within the plane of the bilayer.

Many physical and functional properties of biological membranes depend on the particular way in which the various components of the membrane are organized and distributed within the bilayer (Thompson & Huang, 1978). Furthermore, changes in the equilibrium distribution of membrane components have been associated with a variety of biological phenomena such as cell fusion (Papahadjopoulos et al., 1976), clustering of receptor sites (Taylor et al., 1971), and "capping" and "patch" formation (Raff & De Petris, 1973; Fishman & Brady, 1976). Studies on model membrane systems have demonstrated that the distribution of lipid components along the plane of the membrane is not physicochemically ideal and as such it is characterized by the presence of compositionally different domains (Shimshick & McConnell, 1973; Van Dijck et al., 1978; Correa-Freire et al., 1979). It has also been demonstrated that this distribution can be altered by changes in external variables and by changes in the physical state of the lipid molecules (Wallace & Engelman, 1978). Unfortunately, since a quantitative characterization of the lateral distribution of components in lipid bilayers has been unavailable, it has been impossible to establish quantitative correlations between the dynamic organization and functional properties of biological membranes.

In this communication the results of a computer analysis of the lateral distribution of molecules in phosphatidylcholine mixtures are presented. These semiempirical calculations are based upon the nonideality parameters obtained experimentally from the phase diagrams of these mixtures. These parameters are used to simulate bilayer lattices with a computer, by using a newly developed method. Direct analysis of the comput-

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